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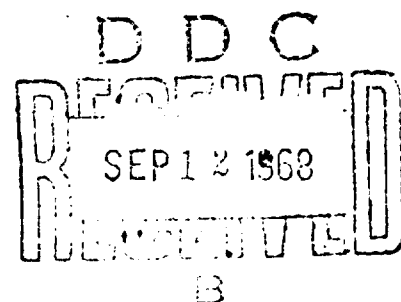
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Soluble Toxins of Some Enterobacteriaceae

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TECHNICAL TRANSLATION

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SOLUBLE TOXINS OF SOME ENTEROBACTERIACEAE

by

I. N. Yanishevskaya

Source: Meditsinskiy Zhurnal Uzbekistana
No 1, 1965, pp 43 - 50

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SOLUBLE TOXINS OF SOME ENTEROBACTERIACEAE

Source: MEDITSINSKIY ZHURNAL
UZBEKISTANA (Russian)
1965, No.1, pp. 48-50

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Uzbek Scientific Research Institute of Epidemiology, Microbiology & Infectious Diseases)

The purpose of this work is to establish the presence of soluble toxic substances in different representatives of the Family Enterobacteriaceae which secrete these toxins during their vital activity.

Therefore, we examined 162 strains, being various representatives of the Family Enterobacteriaceae, of the genera Salmonella, Shigella, Escherichiae.

Cultures were sent from the L. A. TARASEVICH Control Institute and from the laboratories of Tashkent City's Sanitary Epidemic Post.

Toxic substances were obtained by cultivating bacteria on cellophane leaf according to the BIRCH-HIRSCHFELD method (1934), with subsequent centrifugation for 30 minutes (10,000 r.p.m.).

Soluble toxins were discovered almost in all representatives of the Family Enterobacteriaceae (Salmonella, Shigella, Escherichiae). B. faecalis alkaligenes (5 strains) did not form toxin at all. The strongest were the toxins of Sh. Griogor'yeva-Shiga, Sh. Lergia-Sachs (Q771, Q902), S. paratyphi B, whose individual series contain 200 MLD in 1 ml of toxin. It was also established that diverse series of the toxin detected in different strains of one and the same species showed variation in toxicity (2 -20--30--200 MLD in 1 ml of toxin).

All toxins were characterized by clearly marked thermostability: --- their heating at 100°C temperature for 2 - 3 hours did not reduce their toxicity, and all animals contaminated with them died. Toxicity was lost only after a 30-minute autoclaving at 120°C.

The toxins were not dialysed, precipitated by trichloroacetic acid (pH=3, 5), saturated ammonium sulfate solution, acetone, and 90° ethanol.

Attempt to get toxoid ended in failure.

The immunogenic properties of toxins were verified in the following way. Increasing toxin doses in dilutions 1:80, 1:20, 1:10, 1:2 MLD per 0.5 ml were administered to white mice subcutaneously at 3 - 5 days' intervals. We tested samples of toxins obtained from S. typhi, S. paratyphi A, S. paratyphi B, Sh. Grigor'yeva-Shiga, Sh. Schmidt-Stutzer, Sh. Largia-Sax (serotypes Q₇₇₁, Q₉₀₂, Q₄₅₄, Q₁₀₃₀, Q₁₁₆₇), Sh. Flexner (serotype C), Sh. Newcastle, Sh. Boyd-Novgorodskiy (serotype III), Sh. Kruze-Sonne.

Each toxin sample was administered to 20 white mice. The immunity was checked 10 days after the last immunization with intraperitoneal administration of native toxins containing 2--20--80--200 MLD per 0.5 ml.

As a result of the tests, it was established that active immunization helped the survival of inoculated mice, while all animals of the control group died.

Thus, toxic products obtained on cellophane leaves from different representatives of *Salmonella* and *Shigella* possess immunizing properties.

The antigenic properties of soluble toxins were checked in precipitation reactions (by the usual modification and by the diffusional variety in gel according to WUCHTERLOHN. In the tests, antimicrobial and antitoxic sera of Sh. Grigor'yeva-Shiga, Sh. Largia-Sax (serotype Q₇₇₁) were used which were prepared in our laboratory.

The titres of antimicrobial sera were the following: --- Sh. Grigor'yeva-Shiga 1:6400, Sh. Largia-Sax Q₇₇₁ 1:800, antitoxic serum of Sh. Grigor'yeva-Shiga 1:1024, Sh. Largia-Sax Q₇₇₁ 1:512. For antigens we used soluble toxins of the listed representatives of the group of bacteria as well as a combined antigen obtained according to the method of BUAVERA for Sh. Largia-Sax Q₇₇₁.

In the arranged reactions, the heterogeneity of soluble toxins and the presence of general antigenic complexes was distinctly determined. Of very great interest are negative results of the reactions with the BUAVERA Sh. Largia-Sax Q₇₇₁ antigen and with the homologous antitoxic serum. At the same time, tests were arranged with the use of a specific antimicrobial Sh. Largia-Sax Q₇₇₁ serum which gave positive reaction with the appearance of one precipitation line according to WUCHTERLOHN. These observations prove that the complex Sh. Largia-Sax Q₇₇₁ (S form) antigen which was extracted according to BUAVERA's method is different antigenically from the soluble toxin obtained from the same strain at its cultivation on cellophane leaf.

Finally, we studied cytopathological changes in the organs and tissues of killed laboratory animals killed by the soluble toxins of various species of the Family *Enterobacteriaceae* (Sh. Grigor'yeva-Shiga, Sh. Largia-Sax serotypes Q₇₇₁, Q₉₀₂, Q₄₅₄, Q₁₀₃₀, Q₁₁₆₇, Sh. Flexner serotype C, Sh. Newcastle, Sh. Boyd-Novgorodskiy serotype III, Sh. Kruze-Sonne, S. paratyphi B). For this purpose, toxins of different concentrations (100 MLD, 1/2 MLD, 1/80 MLD) were administered intravenously to 78 rabbits and intraperitoneally to

260 white mice. No specific differences were found in the effect of soluble toxins obtained from different Enterobacteriaceae upon the organism of animals.

The introduction of 100 MLD toxin to rabbits was associated with an increase in temperature, frequency of respiration, with convulsions; death followed after 18 to 24 hours. Injections of $\frac{1}{2}$ MLD toxin of Sh. Grigor'yeva-Shiga, Largia-Sax, Flexner, Newcastle, Boyd-Novgorodskiy, Kruze-Sonne, S. paratyphi B led to the appearance of paresis and paralysis of the rear extremities in 18 rabbits out of 26 contaminated animals, and in 32 white mice out of 65 infected animals. Repeated administration of 1/80 MLD of any of the listed toxins caused development of marked cacheia (20 rabbits out of 26). All white mice, infected with $\frac{1}{2}$, 1/80 MLD, survived. At the autopsy of dead animals, overfilling of the urinary bladder and hyperemia of the internal organs was noted.

Histological examinations did not detect any specific changes as an effect of toxins of various species of Enterobacteriaceae. Depending upon the dose, in individual cases their effect varied only as to the intensity of cell damage.

After the administration of soluble toxins of Sh. Grigor'yeva-Shiga, Largia-Sax, Flexner, Newcastle, Boyd-Novgorodskiy, Kruze-Sonne, S. paratyphi B, in doses of 10 -- 100 MLD, dystrophic changes were noticed in the organs of experimental animals. The greatest changes occurred in the central nervous system, especially in the motor cells of the anterior horn of the spinal cord. This proves the profound damage of motor cells in the spinal cord and pathogenetically it corresponds to the development of paresis and paralysis in the experimental animals.

Thus, in various representatives of the Family Enterobacteriaceae, of the genera Salmonella, Shigella, Escherichiae, which were cultivated on cellophane leaf, soluble toxic substances could be obtained. They are thermostable, do not dialyze, possess antigenic and immunogenic properties. Their neurotoxic effect was also detected.

2 December 1963.

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13. ABSTRACT <p>Various representatives of the Family Enterobacteriaceae, including the genera Salmonella, Shigella, Escherichiae, produce soluble toxic substances during their life. These toxins are able to produce various damages, even death, in the experimental animals, depending upon the amount of administered dose. Experiments on rabbits and white mice proved that the toxins are thermostable, and they have antigenic and immunogenic properties, but they are not suitable for toxoid preparation. They have neurotoxic, paralytic effect upon motor cells of the anterior horn in the spinal cord.</p>			

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